H

16 Project No. 1713
Book No. 18007 TITLE

Exhibit H, pg. 1 of 20

From Page No. 15

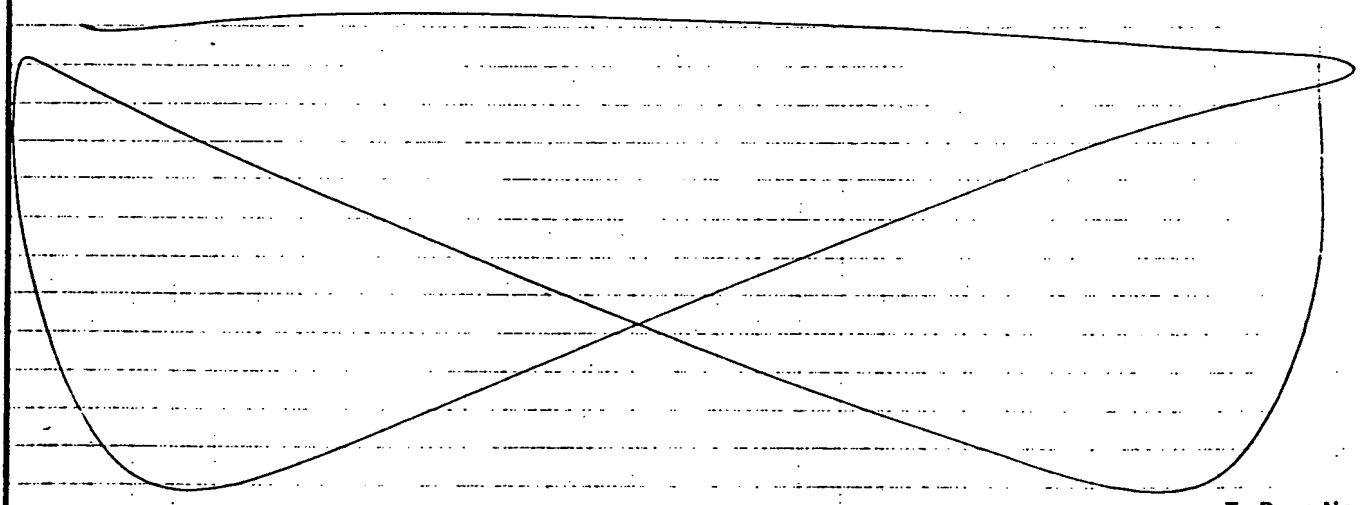
Want to try a serum free (PS04) run on CHO cells to check for Fus expression levels

Have 3 sets of plates currently plus 6 well dishes

Replaced media on 6 well dishes w/ PS04 → Inc

FOR CHO → needs Insulin (bovine)
transferrin
Lipid
trace elements
HEPES

Inc 37°C →



To Page No. 17

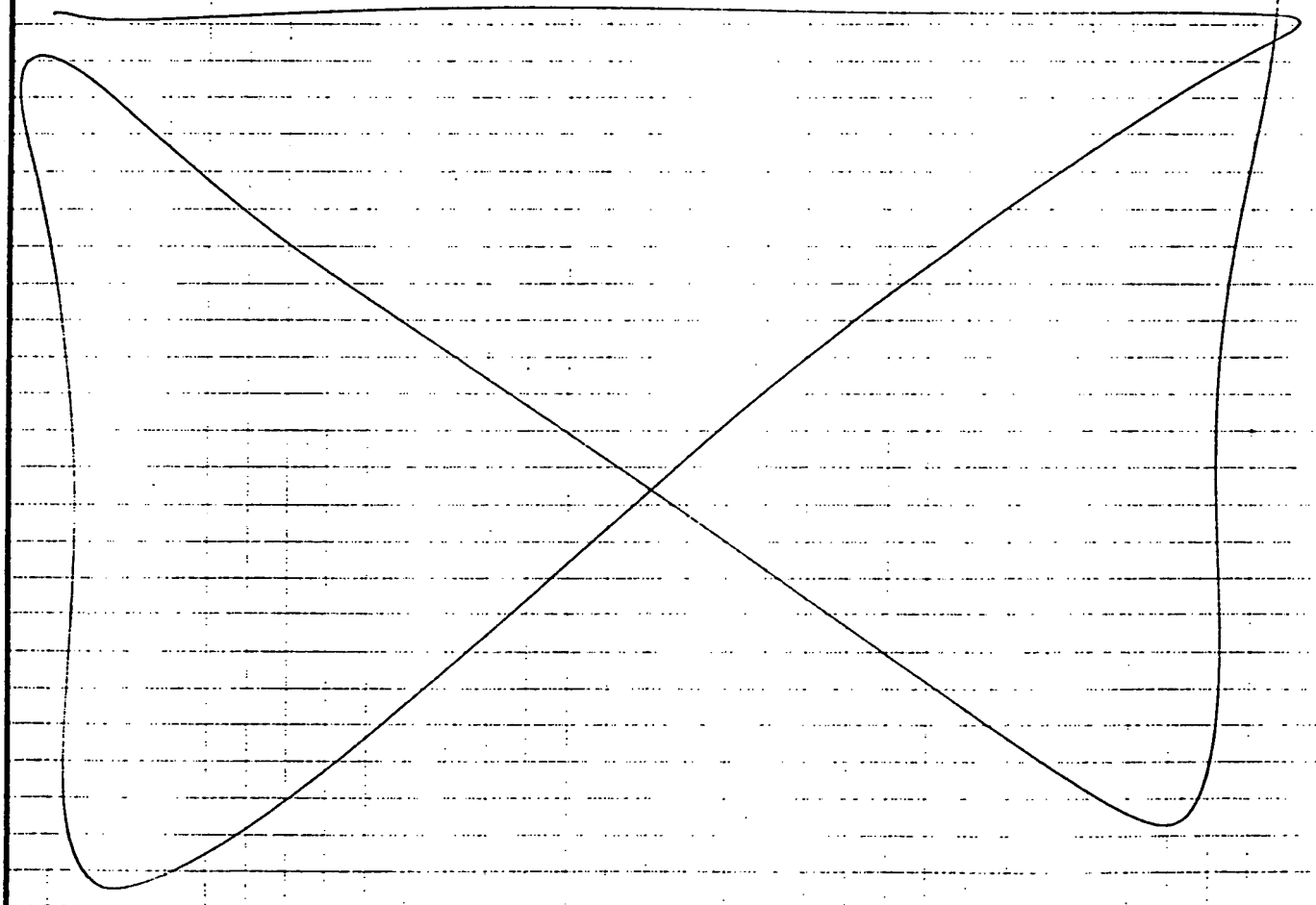
Witnessed & Understood by me,	Date	Invented by / Recorded by Will Bacon	Date THURS 6/17/93

From Page No. 16

Froze down 1 set of FHS picks in serum + 7% DMSO -70°C.

Split 10cm plates each 1:10.

Cont PSO4 on 6 well dishes



17

To Page No. 18

Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by Wm Barron	6/18/93

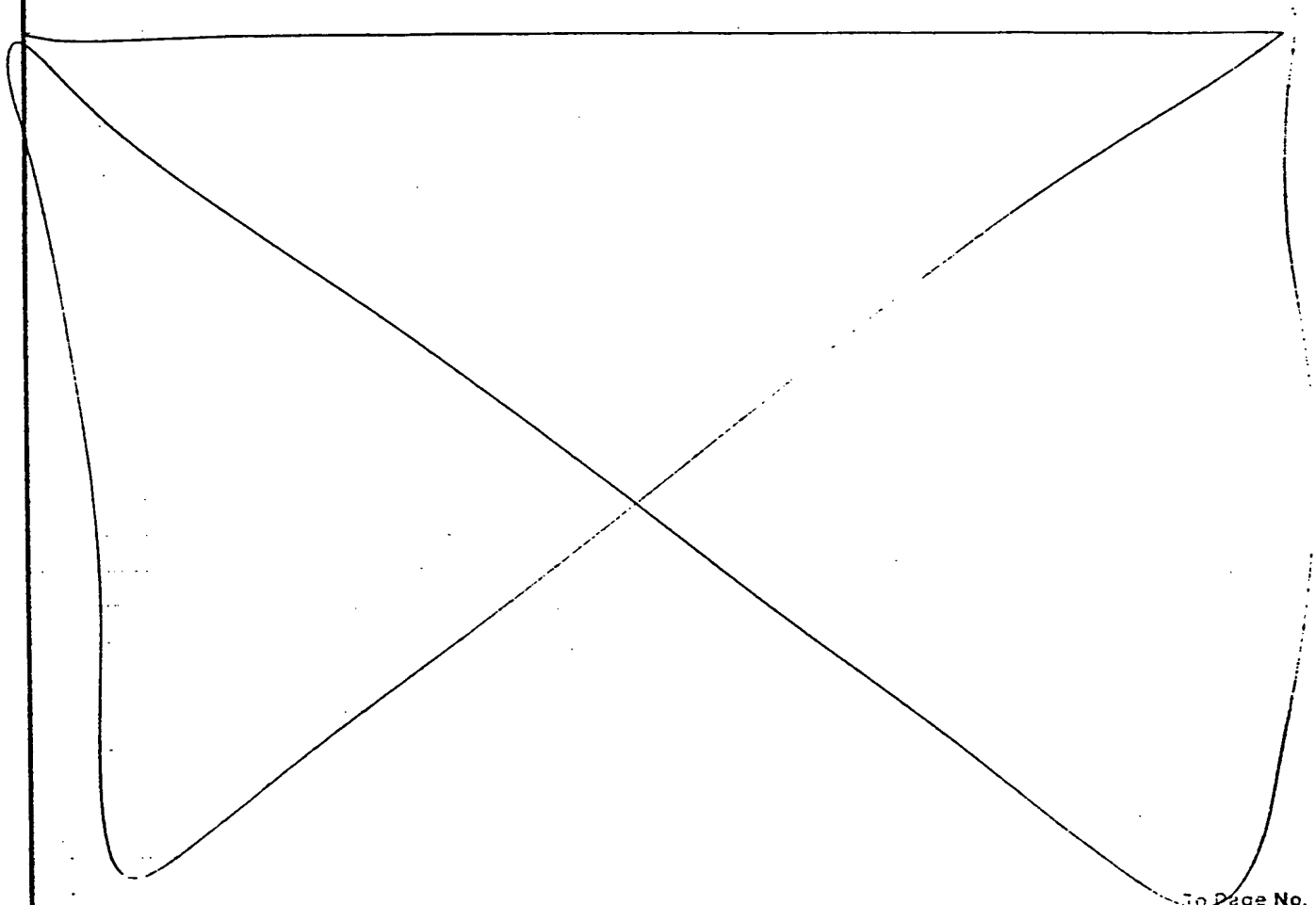
Project No. 1713
Book No. 18802 TITLE

18

From Page No. 17

Harvested PSOY media's
Filtered each Through syringe 0.45μM
Stored -20°C O/N

Split all 36 FUS picks.



To Page No.

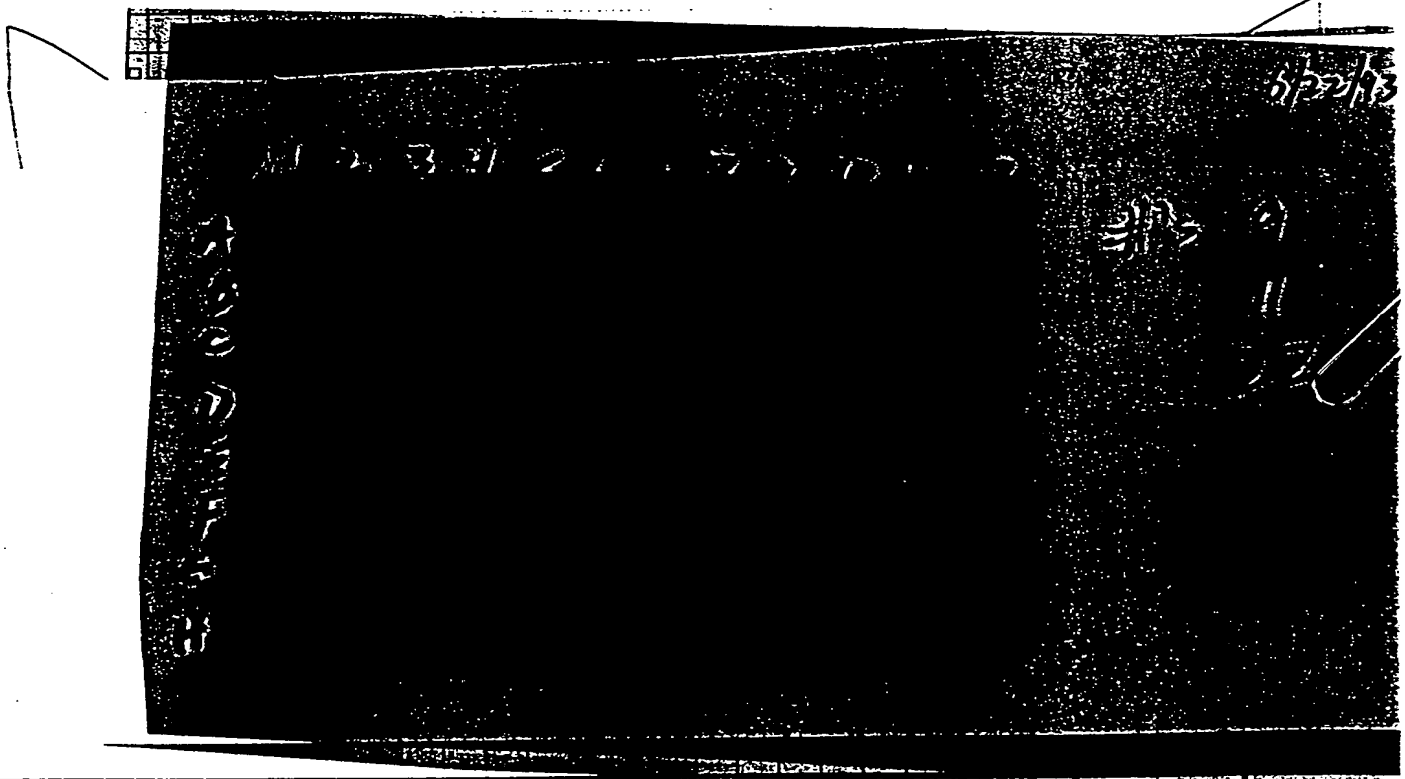
Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by Will Bacon	6/21/93

Project No. 1713

TITLE _____ Book No. 18002 19

From Page No 12

Dot Blotted 200µl each PSOY media
Decorated blot w/ 2 Human Fc
ECL Detected
#'s 9, 11 + 22 appear to have highest expression → discarded all cultures except 9, 11 + 22.
Ready to scale-up for Protein production



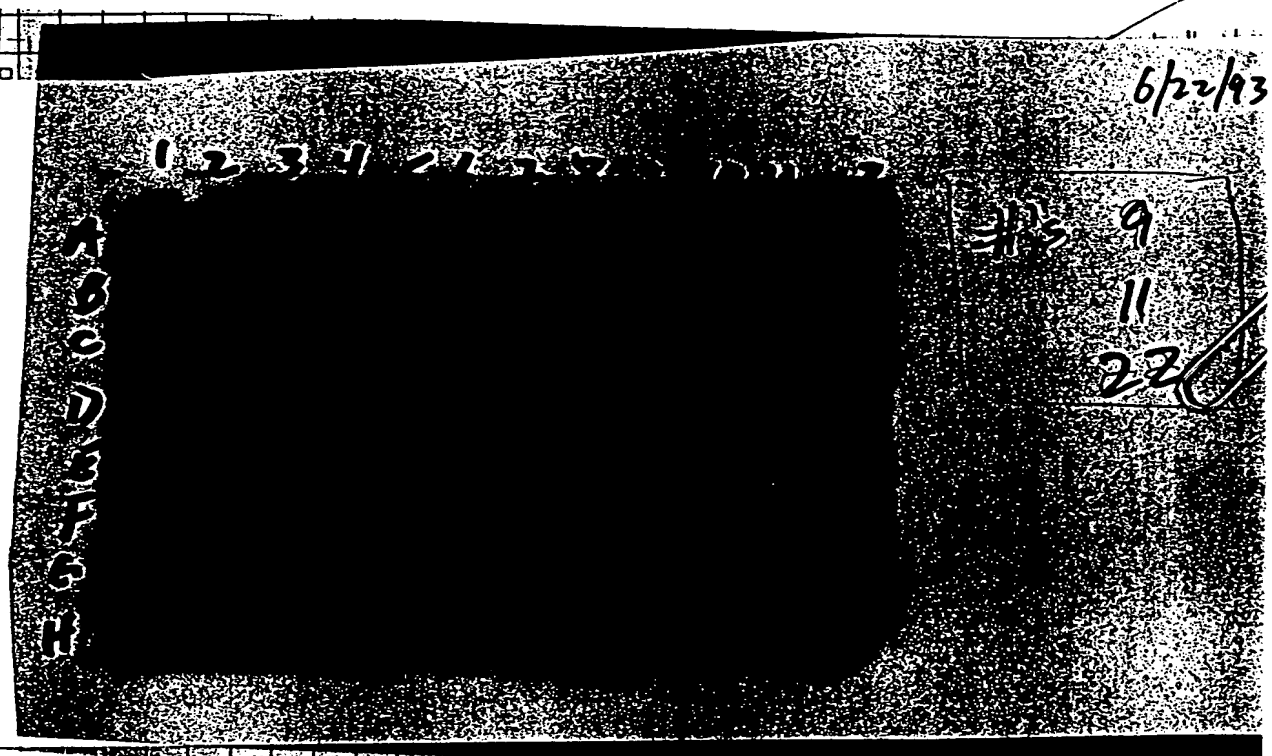
9

Witnessed & Understood by me.	Date	Invented by Will Bacon	Date TUES 6/22/93
		Recorded by	

TITLE

From Page No. 12

Dot Blotted supernatant from media
Decorated blot w/ x Human Fc
ECL Detected
#'s 9, 11 + 22 appear to have highest expression → discarded all cultures except 9, 11 + 22.
Ready to scale-up for protein production



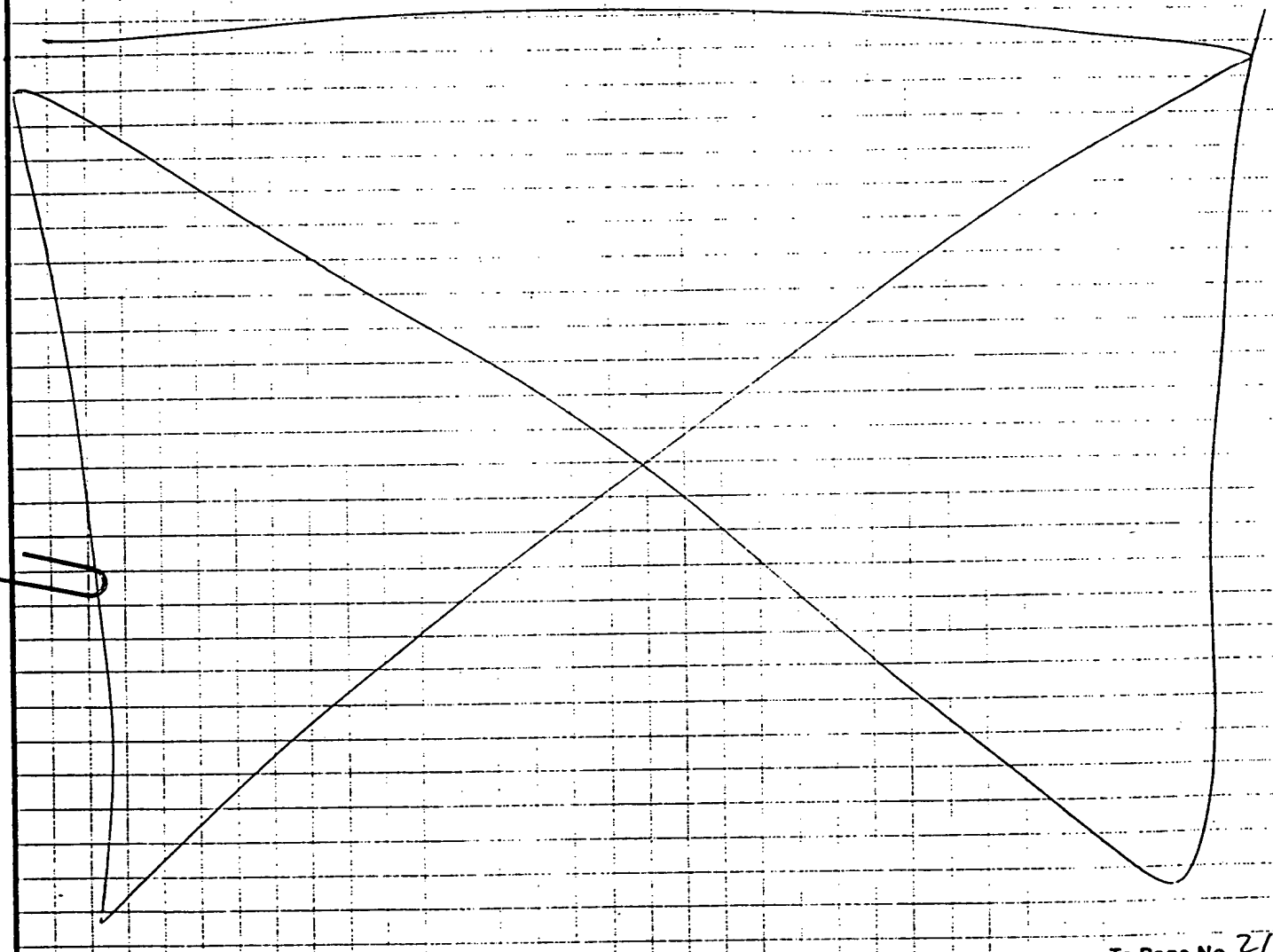
19

Witnessed & Understood by me.	Date	Invented by Bill Bowen	Date TUES 6/22/93
		Recorded by	

Project No. 1713
20 Book No. 18002 TITLE

From Page No. 19

Split all FHS samples 1:10 in 10cm dishes
(9, 11 & 22)
& 1:5 on 15cm dishes (~~FHS~~ #9 only)



To Page No. 21

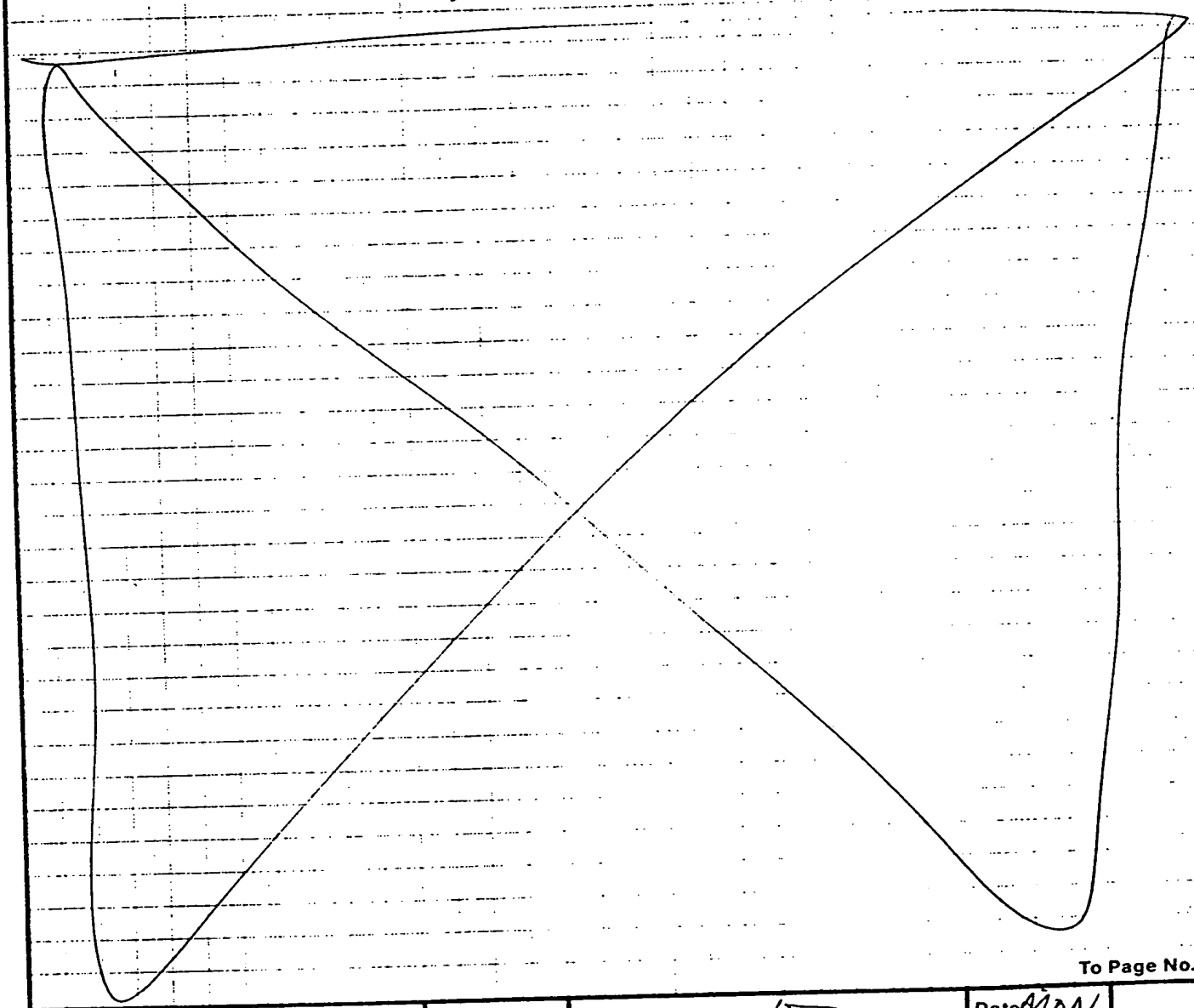
Witnessed & Understood by me,	Date	Invented by	Date FRI 6/25/93
		Recorded by WMBacon	

Project No. 1713
Book No. 18002

TITLE

From Page No. 20

Split Fns 11 + 22 each 1:10 on 10 cm dishes.
Split Fns 9 15cm plate to 5 × 15cm plates
Getting ready for 1st PSO4 run.



To Page No. 2-

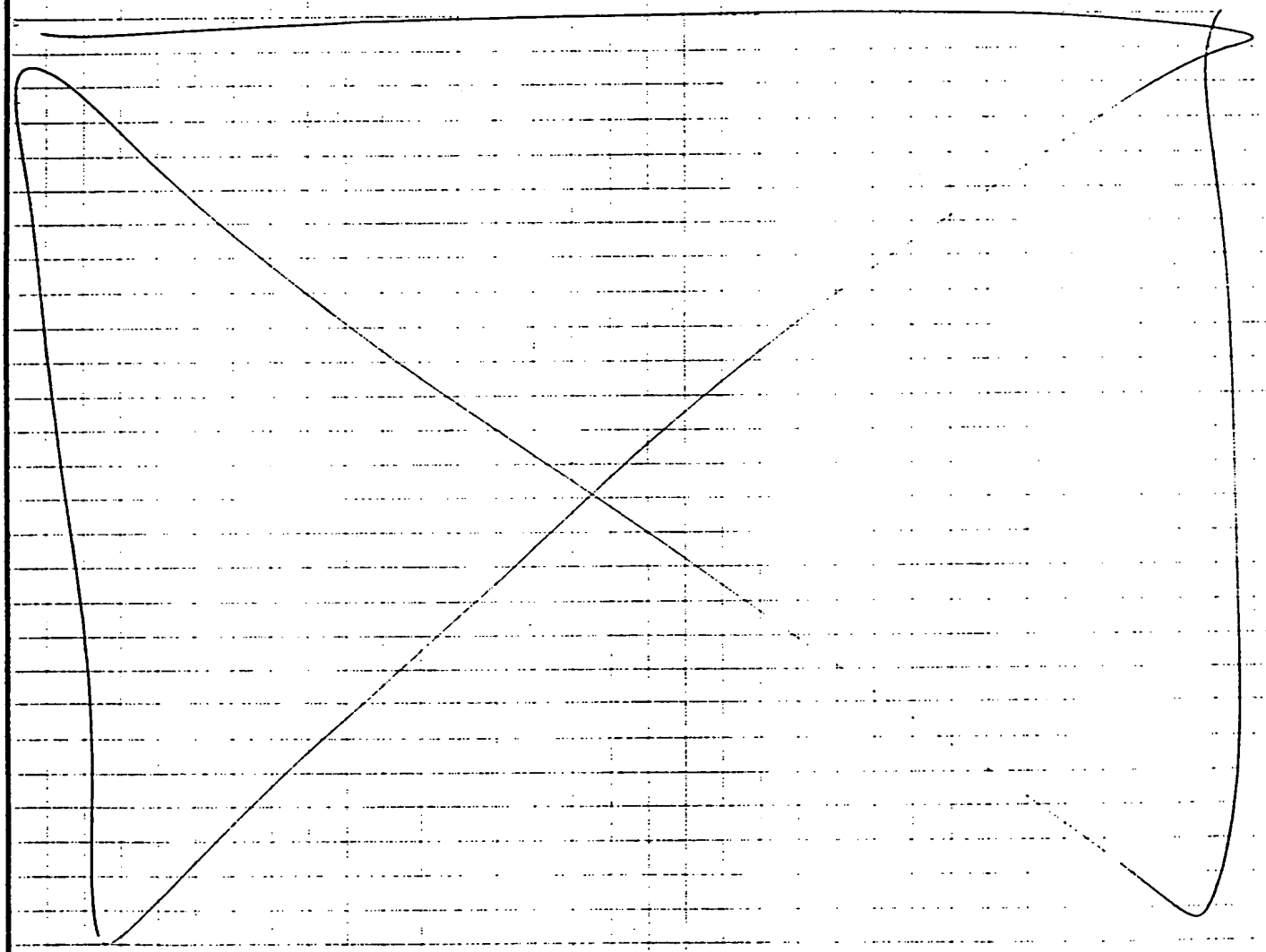
Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		W.M. Bacon	MON 6/28/93

22 Project No. 1713 U
Book No. 18002 TITLE

From Page No. 21

Split each FUS 9 15cm plate to 8 x 15 cm plates
(= 5 x 8 = 40 total)

Inc o/N 37°C 5% CO_2



To Page No. 23

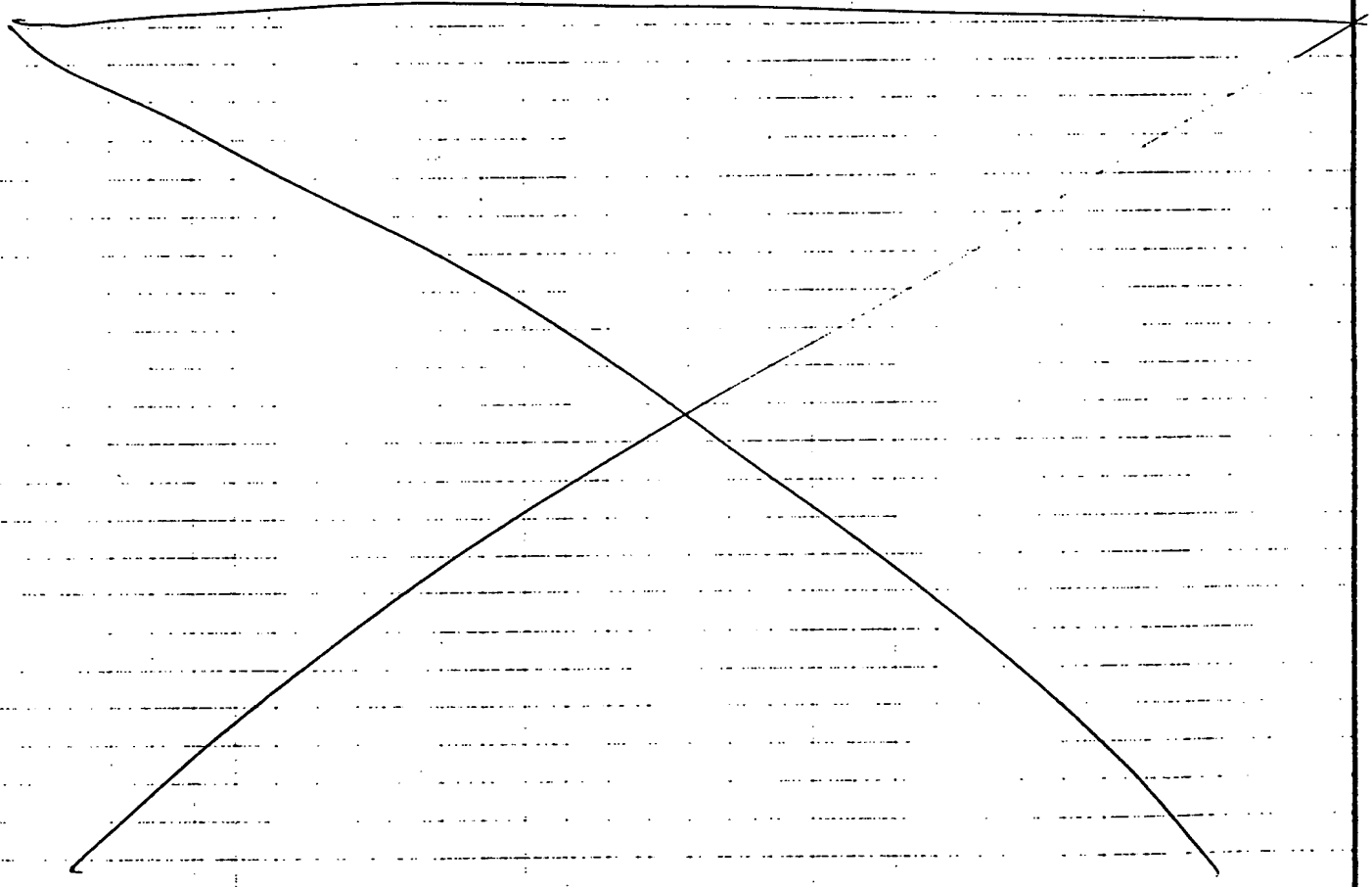
Witnessed & Understood by me,	Date	Invented by	Date WED
		Recorded by Will Bacon	6/30/93

From Page No 22

Changed media on 40 × 15 cm FUS 9 plates to PSO4 → Inc 37°C

Will harvest in ~ 4-6 days (when cells lift off)

Started FUS 9 in 250 ml spinner flask (with 1 entire 16 cm dish worth of cells).



To Page No. 24

Witnessed & Understood by me,	Date	Invented by WM Bacon	Date 5/4/93
		Recorded by	

Project No. 713

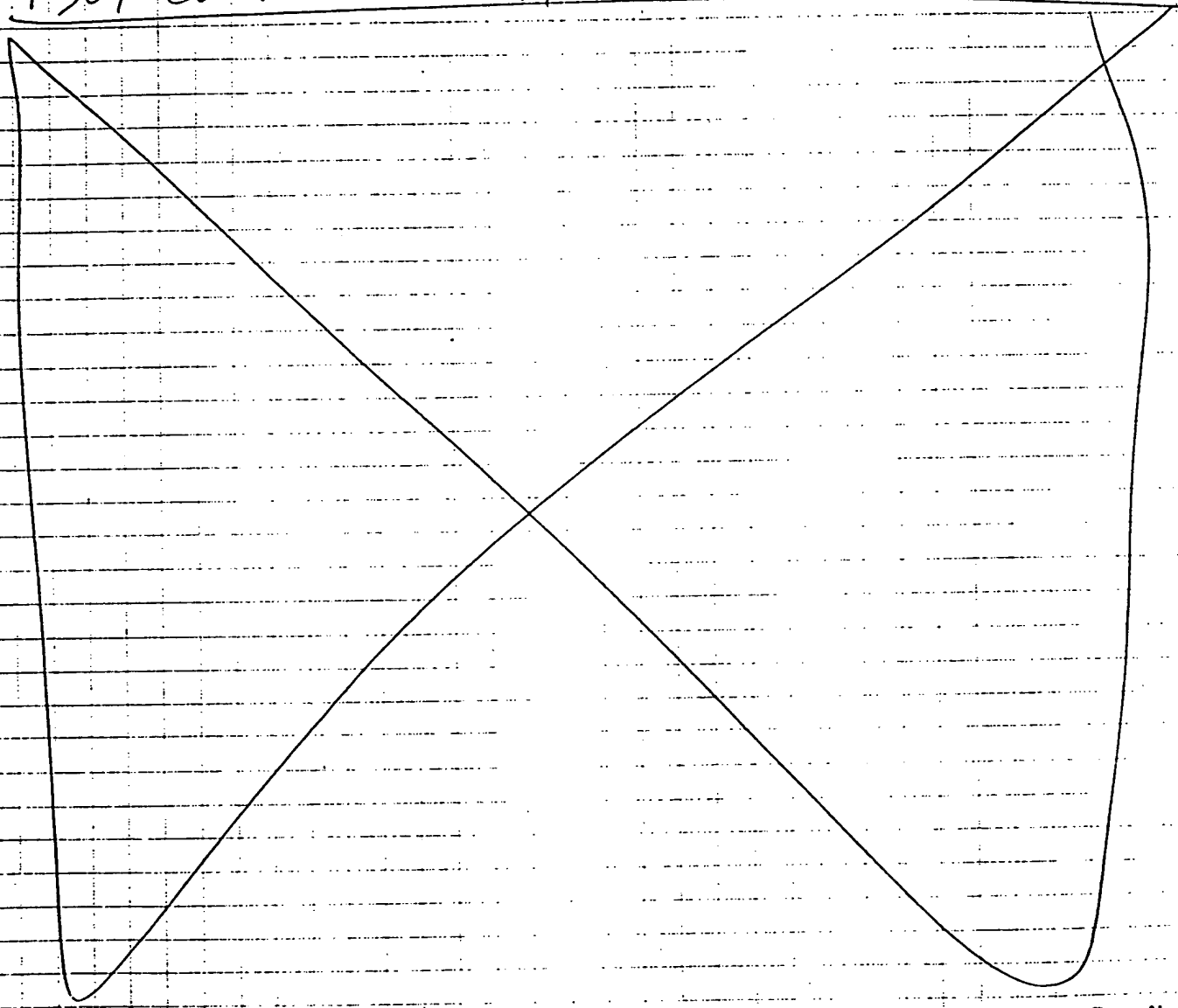
-24 Book No. 18002 TITLE

From Page No. 23

Split Fus 9, 11 + 22 all 1:10

Checked Fus 9 spinners → split 1:10

f504 cont until 7/7



To Page No. 25

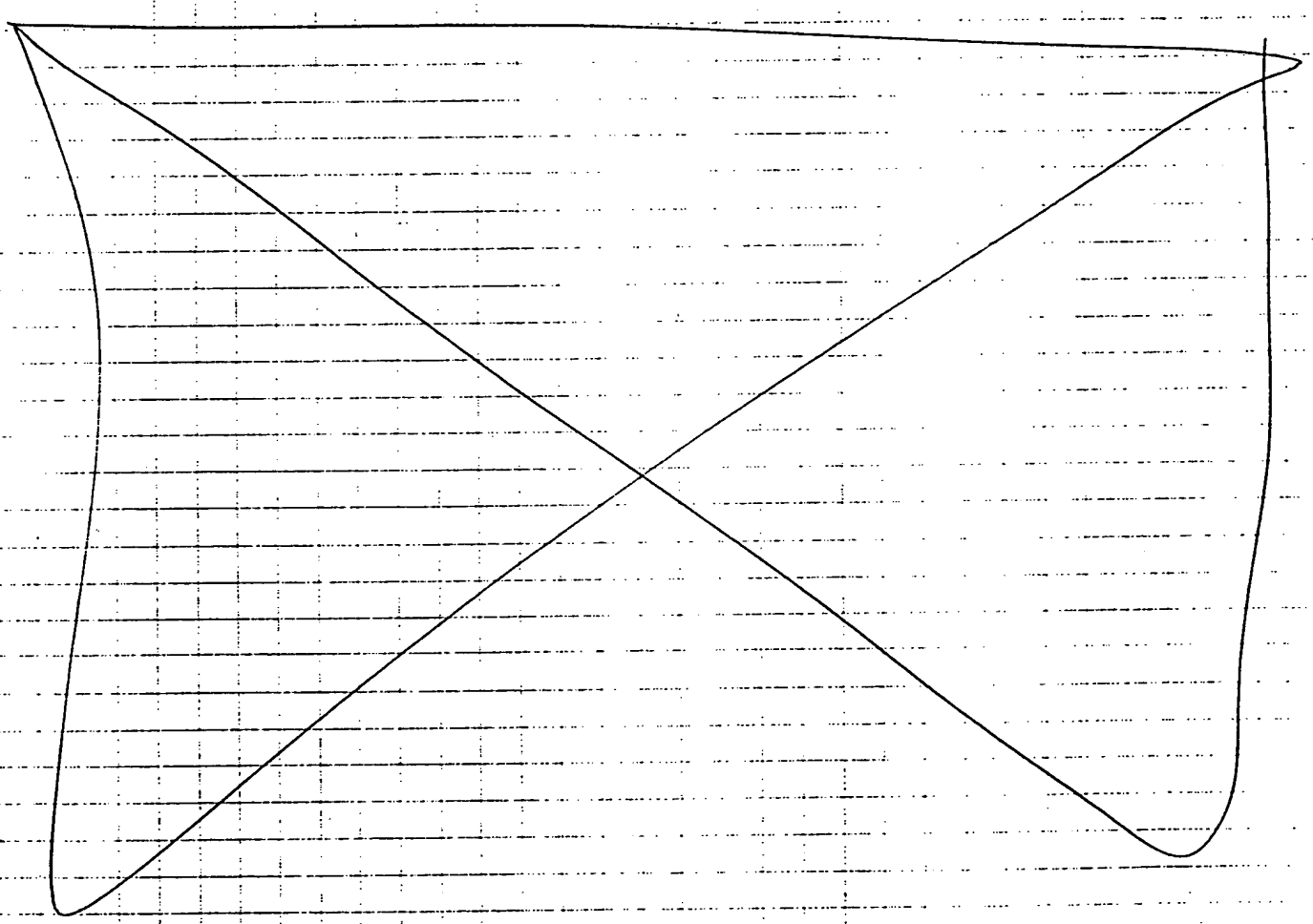
Witnessed & Understood by me,	Date	Invented by / Recorded by Will Bacon	Date TUES 7/6/93

Project No. 1713
Book No. 18002

TITLE

From Page No. 29

Harvested PSO4 media
Filtered Through 0.45μM filter
Added protease inhibitors (PMSF, aprotinin, leupeptin & pepstatin).
Stored 4°C O/N.



To Page No. 26

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		[signature]	WED 7/7/93

From Page No. 25

Protein A column procedure:

Column is washed w/ 0.1M Na Citrate pH 6.0

Sample is made up to 0.1M Na Citrate pH 6.0 filtered & then loaded @ ~4ml/min

after loading column is washed w/ citrate

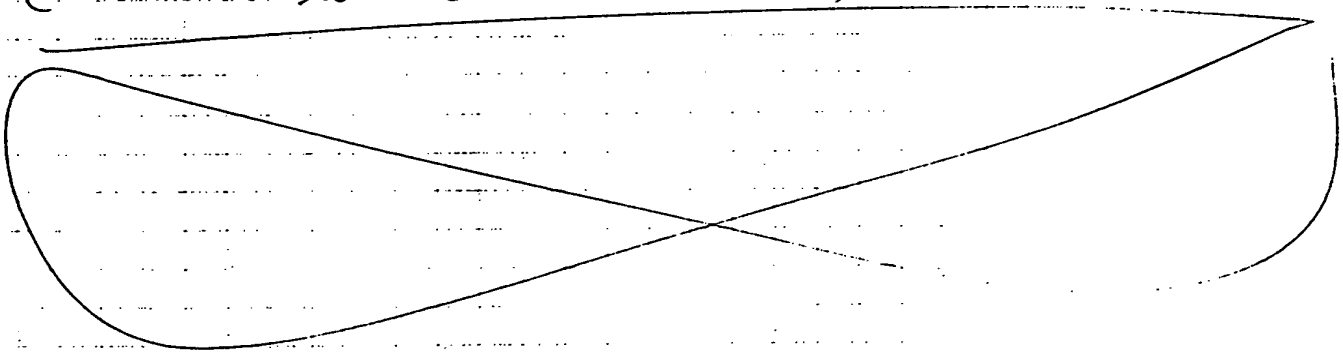
→ when baseline level is reached

then sample is eluted w/ MgCl2 elution buffer (see lab protocols)

Sample is desalted on a PD-10 column & concentrated w/ S+S unit.

Ran entire 1st PSO4 batch, washed, eluted, desalted & stored 4°C c/n

(Volume is ~3.5ml in PBS)



To Page No. 27

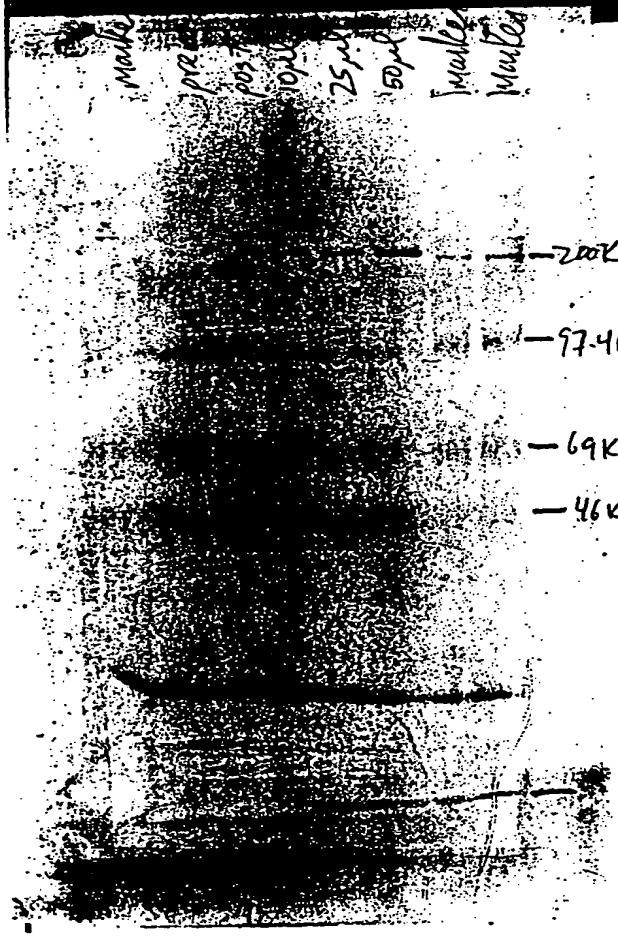
Witnessed & Understood by me,	Date	Invented by Will Bacon	Date THURS 7/8/93
		Recorded by	

From Page No. 26

Ran samples of Pre Prot A, Post Prot A & of recovered material on 10% SDS-PAGE in duplicate for analysis

MW	MW	Sq/ml rec	25µl rec	10µl rec	5µl lost	Sq/ml re	MW	MW	MW	Sq/ml rec	25µl rec	10µl rec	5µl lost	Sq/ml re	MW
16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Cut gel into 2 halves → WESTERN transferred one half & Ponceau'd → other half coomassie stained



(on 100µl of 3.5ml)

Did BCA for protein concentration determination

A562 recovered FHS 9 = 0.192
(see standard curve p. 28)

~~Correlation Coefficient 0.60091~~

~~R²~~

$y = 29.27(0.192) - 0.60091$

$y = 5.02\ \mu g$ in 100µl

$= 50\ ng/\mu l$

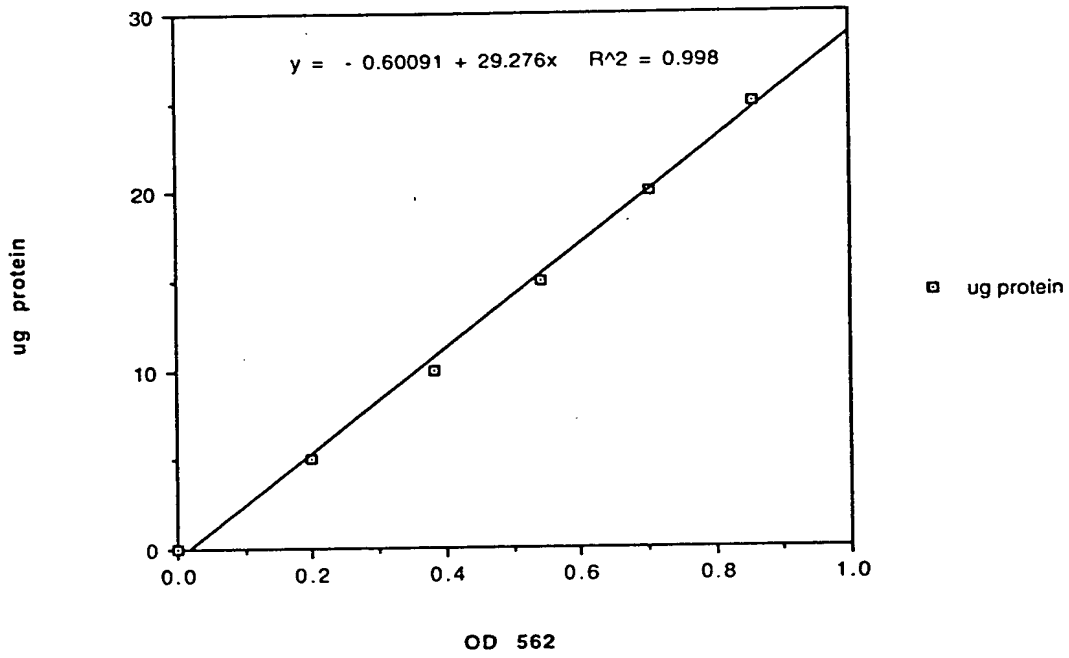
$= 175\ \mu g$ total yield!

To Page No. 28

Invented by
Recorded by WWBaron
Date FRI 7/9/93

17-13
1500 2

27



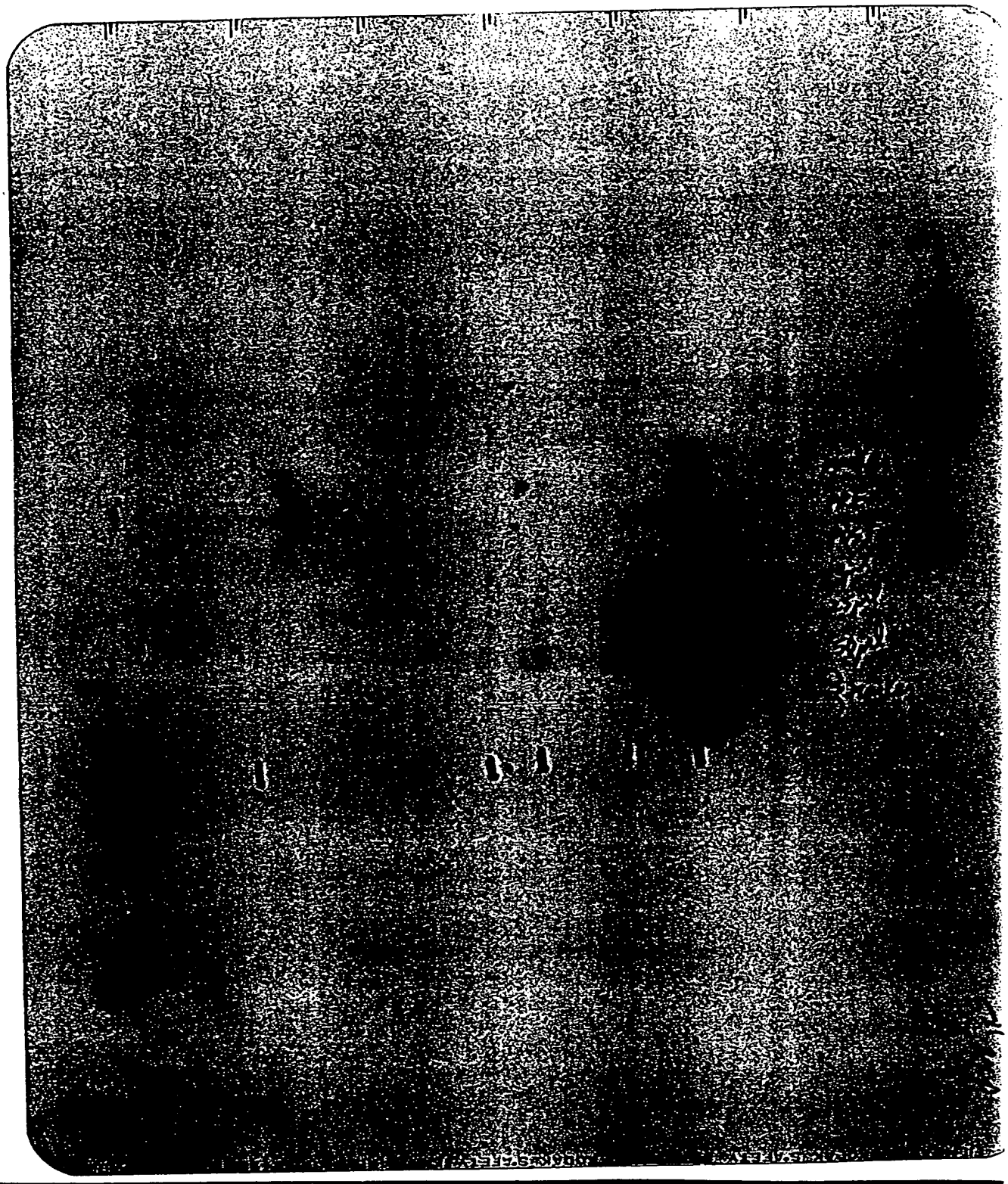
I think that I will get much better yields from spinner culture.

Split Fus 9, 11 & 22 plates all 1:10

Split Fus 9 spinner 1:10

7

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by HWL Bacon	7/9/03

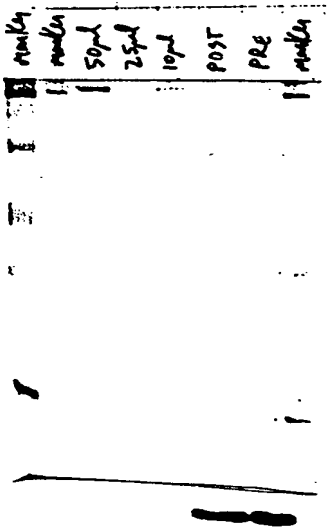


Decorated FUS 9 western w/ α Human Fc

ECL detected — Many species observed including some probable degradation produc

Photographed coomasie stained portion of gel

here major material is seen at ≥ 200K



FUS 9

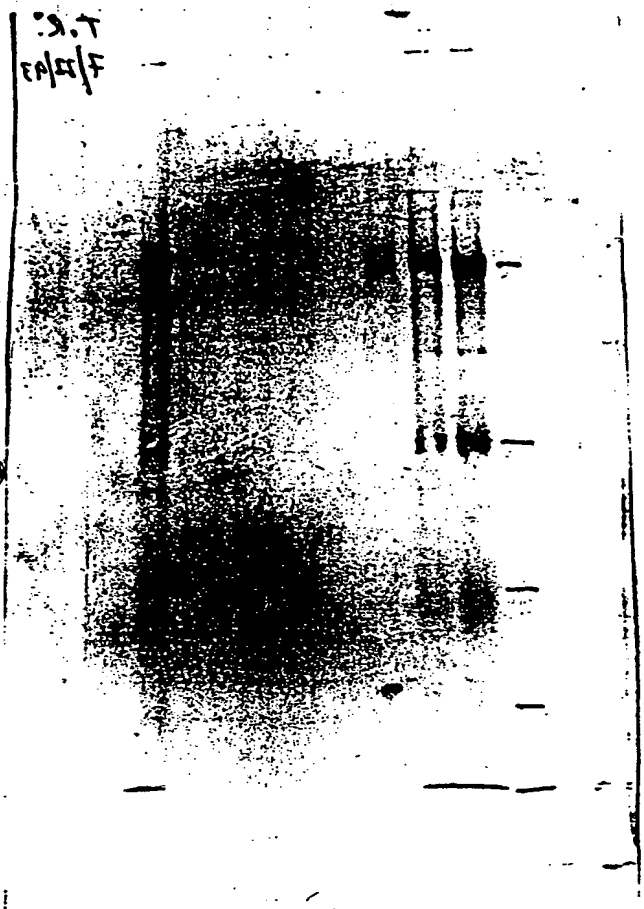
WESTERN!

Split all wells + spinner

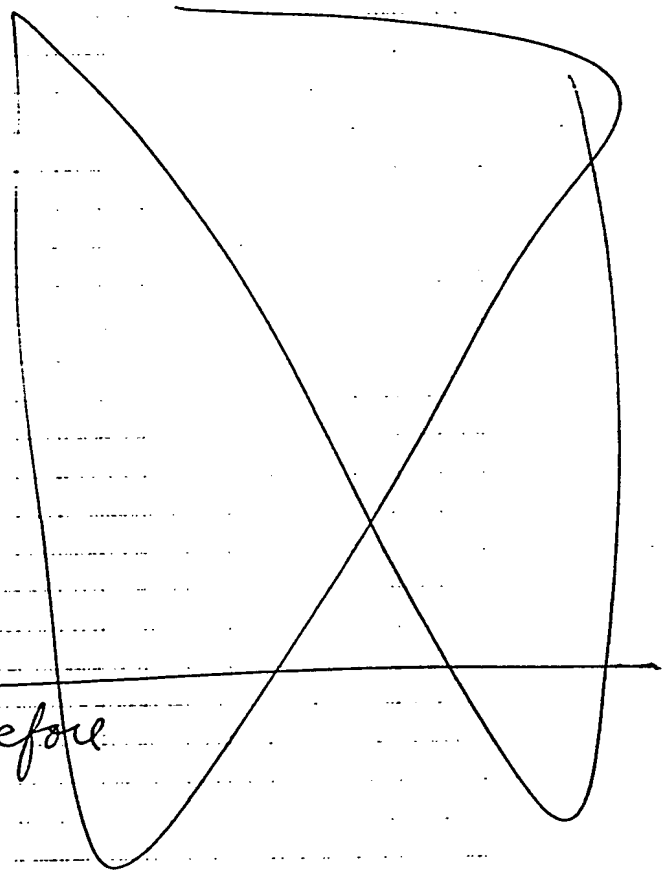
Witnessed & Understood by me,	Date	Invented by WM Barron	Date MON 7/12/93
		Recorded by	

From Page No. 33

Stopped O/N gel → cut into 2 halves
Coomassie Stained ½
WESTERN transferred ½ Other half → Ponceau stained



Stored blot RT
Destained Coomassie O/N



Started PSO4 run #4 as before

To Page No. 35

Witnessed & Understood by me,	Date	Invented by Wm Baron	Date FRI 7/23/93
		Recorded by	

35

Ed

Page No. 36

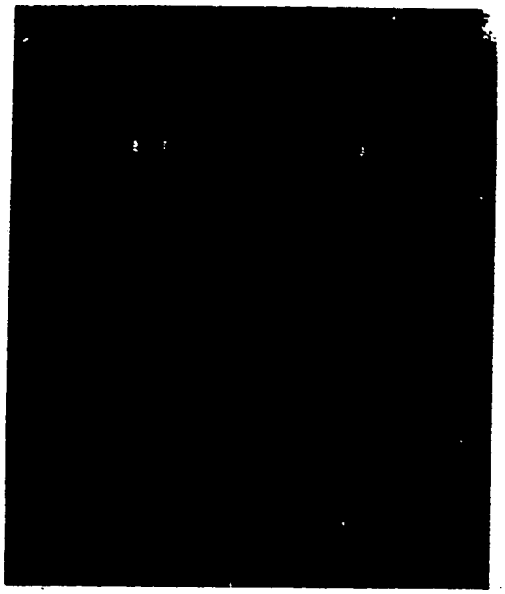
Project No. 1713
Book No. 18002

TITLE

From Page No. 34

Decorated Fusion Western w/ α human Fc → ECL detected

Photographed Coomassie stain



FVS

IgG

ECL

To Page No. 36

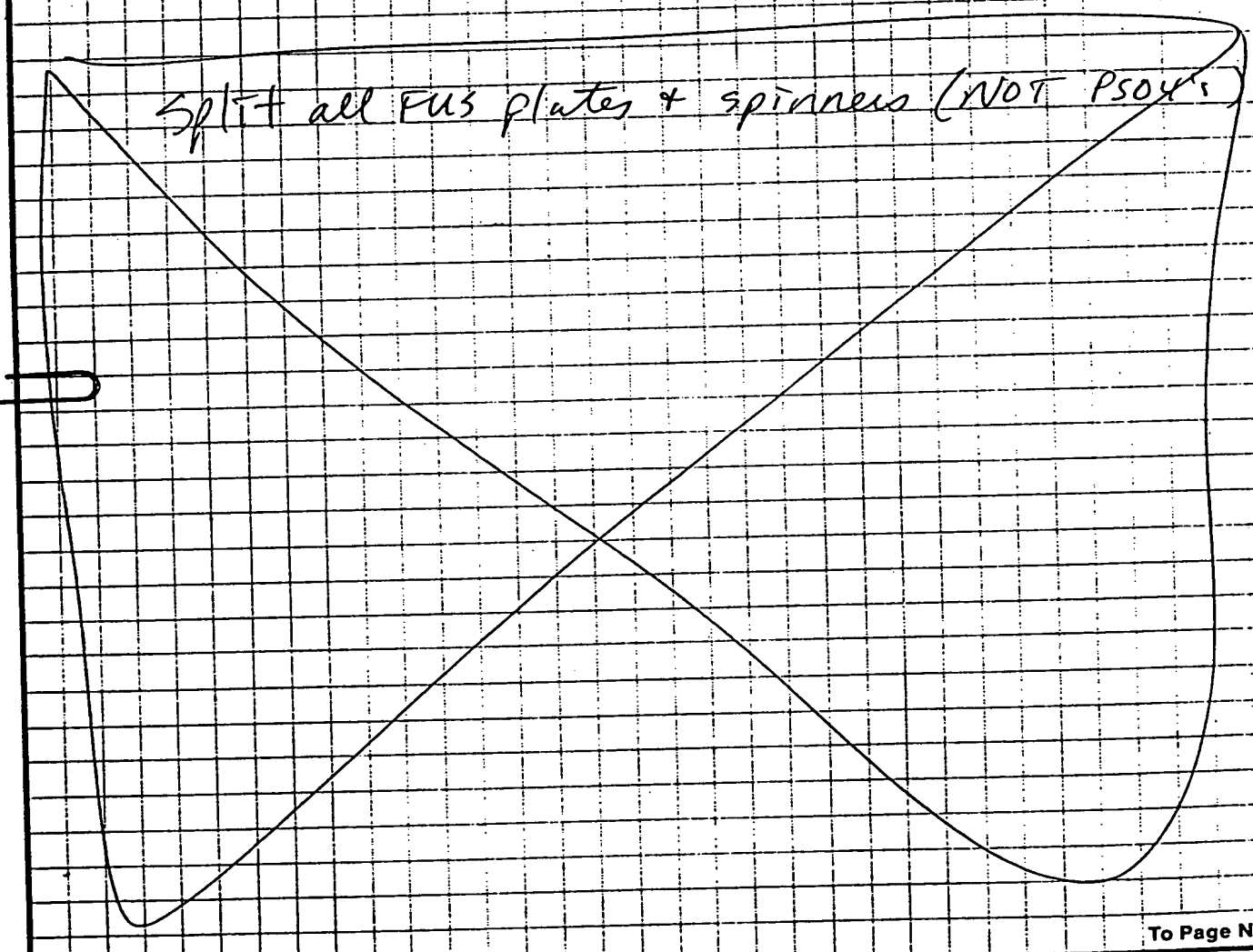
Witnessed & Understood by me,	Date	Invented by	Date MON 7/26/93
		Recorded by WWBaron	

36 Project No. 1713 Book No. 18002 TITLE

From Page No. 35

Harvested 1st PS04 run
Run prot A column
Eluted w/ $MgCl_2$
Desalted, stored 4°C

Split all FUS plates + spinners (NOT PS04's)



To Page No. 37

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		WMBaron	MON 7/26/93